REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested.

The Office Action Summary indicates that claims 8, 19-34 and 36 are pending in the application. In fact, claims 19-34 and 36-38 are under consideration and have been rejected.

By the foregoing amendment, claim 19 has been amended to recite that the adenovirus particles being purified by the claimed method are "infectious" adenoviral particles. Support for this amendment can be found in the specification at least at page 3, line 5. No prohibited new matter has been introduced by way of the above amendment. Applicants reserve the right to file a continuation or divisional application on subject matter canceled by way of this Amendment.

Rejections under 35 U.S.C. § 103

The previous rejection of claims 19-38 under 35 U.S.C. § 103(a) for allegedly being unpatentable over Shabram et al., WO 96/27677 A2 ("Shabram"), in view of Berg, WO 98/33572 A1 ("Berg"), and Bondoc et al., *J. Indust. Micro. & Biotech.*, 20:317-322, 1998 ("Bondoc"), and Blanche et al. WO98/00524 (represented in English by U.S. Patent No. 6,458,958, "Blanche"), has been withdrawn. However, claims 19-34 and 36-38 now stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Shabram in view of Berg, Bondoc, and Georgiou et al., U.S. Patent Number 6,027,888 ("Georgiou"). This rejection is respectfully traversed.

The rejection set forth in the Office Action mailed March 30, 2006 is substantially as stated in the Office Action dated December 15, 2004 and in previous Office Actions with respect to Shabram, Berg, and Bondoc, only the citation of Georgiou is new.

The amended method claims are now directed to a method for purifying infectious adenoviral particles from a crude viral preparation comprising a combination of two chromatographic steps, a fluidized bed anion exchange chromatography that is carried out on particles of adsorbent having the recited structural characteristics (comprising an agarose matrix and a central core comprising quartz, and dextran chains covalently coupled to the agarose matrix on which are attached positively charged groups) and a gel filtration chromatograph that is carried out on a support comprising an alkyl dextran/methylene bisacrylamide matrix or an ethylene glycol/methacrylate matrix.

The Office acknowledges that Shabram does not teach all the elements of the claimed method by not disclosing the step of gel filtration and the specific type of chromatographic materials as instantly claimed. *See* OFFICE ACTION at 3.

Shabram is drawn to a method of purifying recombinant adenovirus particles from a cell lysate comprising two chromatographic steps, either IEC followed by IMAC or IEC followed by HIC.

In an attempt to correct the deficiencies of Shabram, the Office relies upon Berg, Bondoc, and Georgiou. The Berg reference reviews fluidized bed chromatographic techniques for separating "macromolecules" from a liquid sample using particles of adsorbent comprising a ligand having affinity to the molecule being purified linked to an agarose-quartz matrix via flexible extenders. Bondoc discloses a method for purifying adenoviral particles by gel filtration chromatography (page 318, first column). Lastly, Georgiou teaches that chromatographic material comprising alkyl dextran cross-linked with methylene

bisacrylamide can be used for the purification by gel filtration chromatography of recombinant proteins produced in bacterial cells (column 38 lines 44-65). The Office alleges that it would have been obvious to use the adsorbent particles taught by Berg in Shabram's method and that one skilled in the art would have been motivated because Berg's method is aimed at improving total yields and productivity in adsorption processes in fluidized beds. *See* OFFICE ACTION at 4-5. The Office further alleges that its would have been obvious to use the material taught by Georgiou in the method of Bondoc and to combine these with Shabram and Berg. *Id.*

However, the prior art fails to provide the required teachings or suggestion to support a proper prima facie case of obviousness. To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Third, the prior art reference (or references when combined) must teach or suggest all the claim limitations. M.P.E.P. § 2143. Applicants submit that in the present case neither the requisite motivation nor reasonable expectation existed to make the claimed combination.

Shabram fails to teach or suggest that the fluidized bed technique would work for purifying adenovirus particles or provide any indication that there would be a reasonable expectation of success in trying such a method. Shabram only mentions fluidized bed methods in a recitation of the general variety of techniques available in the art for IEC chromatographic separation in passing, *i.e.*, conventional packed bed (*i.e.*, gravity), high pressure liquid using radial or axial flow, batch and fluidized bed (page 9, lines 13-15). Shabram provides no teaching that would lead a person of skill in the art toward the present

invention. The working examples of Shabram's methods are directed **exclusively** to conventional packed bed chromatography. Mere knowledge of fluidized bed chromatography techniques could not simply be translated into predictability of success. Thus, Shabram does not provide any incentive to the skilled person to the specific choice of fluidized bed anion exchange chromatography for purifying infectious adenovirus particles.

Berg does not remedy to the deficiencies of Shabram. Berg does not mention whatsoever that the described fluidized-bed technique could be effective for virus purification, and even less for adenovirus purification. Quite to the contrary, Berg teaches that the described fluidized technique will function for the separation of macromolecules such as polysaccharides, proteins, polypeptides, nucleic acids and synthetic water-soluble polymers.

Adenovirus particles are entirely distinct from the type of the macromolecules described by Berg (*i.e.*, polysaccharides, proteins, polypeptides, nucleic acids and synthetic water-soluble polymers). Indeed, adenovirus particles are extremely complex biological molecules composed of hundreds of polypeptide subunits formed by the association of more than 10 different types of proteins and a nucleic acid genome. *See*, Lennart Philipson, *Structure and assembly of Adenoviruses, in Current Topics in Microbiology and Immunology, Vol. 109,* 10 (1984) (Attached hereto as Exhibit C). The adenovirus capsid normally includes 720 hexon proteins, 60 penton base proteins, 36 fiber proteins, 240 pIX proteins as well as minor capsid proteins that cement the viral structure. Thus, adenovirus particles are orders of magnitude larger and heavier than the macromolecules cited by Berg as suited for fluidized-bed separation, in addition to being far more complicated.

This is further supported by Berg's teachings that the fluidized-bed technique "*is normally **limited** to the adsorption/separation of compounds that have **a molecular weight***

below 1,000,000 Dalton." *See* Berg at 12 (emphasis added). The size of an adenovirus particle is about 150 fold above the upper limit given by Berg.

The Office has asserted that Berg does not discourage a person of skill in the art from modifying the method for higher molecular weight molecules, but only teaches "usual" molecular weights for which the method is used. *See* OFFICE ACTION at 7. Applicants submit that Berg explicitly describes an upper limit for the method and that further discouraging a person of ordinary skill from purifying molecules larger than the explicitly taught upper limit of the technique would have been redundant. The 10^6 Dalton limitation taught by Berg does not correspond to the "usual" molecular weight but to the "upper size limit" beyond which Berg teaches that the fluidized bed is not effective. In its ordinary sense, the term "***limited***" stresses the existence of limits which are not, cannot, or may not be passed over. *See, e.g.*, RANDOM HOUSE DICTIONARY OF THE ENGLISH LANGUAGE, SECOND EDITION UNABRIDGED at 1115 (Exhibit A, attached hereto). Because of the huge difference – over 2 orders of magnitude – between the molecular weight of an adenovirus particle and the upper size limit given by Berg, a person of **ordinary** skill in the art would not have expected the fluidized bed chromatography to be effective for adenovirus purification. At best, a reasonable person of ordinary skill might have tried Berg's method for purifying macromolecules close to the 10^6 Dalton molecular weight limit (*e.g.*, $2x10^6$ Dalton), but not orders of magnitude larger.

Furthermore, the macromolecules taught by Berg are significantly different from adenovirus particles in terms of stability. As reported by Huygues et al., adenoviruses are somewhat fragile. Huygues et al., *Human Genome Therapy*, 6:1403-16 (1995), at page 1404, first column (previously provided as Exhibit B to the Amendment and Reply filed March 30, 2004). It must be recognized that each of the proteins constituting the adenovirus capsid has a role in the integrity of the virus particle and thus contributes to the viral infectivity. For

example, adenovirus binds to its host cell through interaction of fiber trimers to the primary cell receptor and internalization required binding of the penton base to cell integrins. Any purification process must preserve the integrity of each of the capsid proteins in order to result in infectious adenovirus particles.

Moreover, it is well known in the art that anion exchange separation is based on ionic interactions between the negatively charged regions of the molecule being purified and the positively charged ligands attached to the chromatographic material. In this respect, ion exchange interactions are influenced by the net charge as well as the surface charge distribution. *See, e.g.,* Protein Purification, Janson and Ryden, eds. (Wiley 1998) at 148-149 (Exhibit B, attached hereto). Such parameters depend upon the amino acid composition, the folding and the various posttranslational modifications that many proteins undergo (*e.g.*, addition of phosphates in phosphoproteins, gamma carboxylates in gamma carboxylated proteins and sialic acid residues in glycoproteins). One skilled in the art would have anticipated that such parameters are distinct between a macromolecule and an adenovirus particle which exhibits at its surface more than hundreds of proteins (720 hexons+ 60 pentons + 36 fibers + 240 pIX + all minor capsid proteins) interacting each other through ionic, electrostatic and other interactions in order to maintain the virus structure.

The foregoing facts emphasize the differences in predictability of success between macromolecule purification and adenovirus particle purification. The skilled person would not have known or even had a reasonable expectation that infectious adenovirus particles could be successfully purified by fluidized-bed chromatography. Understanding the differences between macromolecules and adenovirus particles, one of ordinary skill in the art would not have looked to the Berg teaching that macromolecules can be purified by fluidized-bed chromatography as suggesting that adenoviruses can be successfully purified

with the same technique. Therefore, the Berg reference, alone or in combination with Shabram, provides no motivation or reasonable expectation of success that the fluidized bed separation taught by Berg for simple macromolecules would in fact work with adenovirus particles.

The above reasons present strong evidence that due to the numerous differences between adenovirus particles and macromolecules, the teachings of Berg are not applicable to the presently claimed method and do not provide any evidence supporting a reasonable expectation of success that Berg's material would have resulted in purified infectious adenovirus particles.

It is impermissible to first ascertain factually what applicants did and then view the prior art in such a manner as to select from the random facts of that art only those which may be modified and then utilized to reconstruct applicant's invention from such prior art. *See, e.g., Interconnect Planning Corp. v. Feil*, 227 U.S.P.Q. 543, 550 (Fed. Cir. 1985); *see also, In re Shuman*, 150 U.S.P.Q. 54, 57 (C.C.P.A 1966). In the present rejection, the Office has proposed the combination of Shabram with Berg, without any teaching or suggestion that would lead one of skill in the art to make the combination and in direct contravention of the teaching of Berg. The proposed combination can only be motivated by impermissible application of hindsight out of a desire to reconstruct the presently claimed invention, where the prior art fails to even suggest the claimed combination.

The Office has further alleged that it would have been obvious to modify Shabram's method by substituting Shabram's step of IMAC with Bondoc's step of gel filtration. The Office contends that one would have had a reasonable expectation of success that the gel filtration step would have resulted in purified adenoviruses because Bondoc reports that the

adenovirus particles obtained by gel filtration were comparable with those obtained with standard CsCl method.

The present claims recite the use of specific chromatographic materials to implement the gel filtration step. Bondoc does not teach at all which chromatographic material will result in successful purification of infectious adenovirus particles. Hundreds of chromatography materials based on natural, synthetic or mixed polymers are available to the public, as evidenced by the enclosed list (from Protein purification). There is nothing in the prior art that would lead one of ordinary skill to select materials comprising ethylene glycol/methacrylate copolymers or alkyl dextran/methylene bisacrylamide out of all the possible materials. Therefore the presently claimed invention is not obvious.

The Office has alleged that one skilled in the art would have been motivated to use the alkyl dextran/methylene bisacrylamide material described in Georgiou (column 38 lines 44-65) for Bondoc's gel filtration, because although Bondoc's disclosure does not detail the specific materials to be used, Georgiou provides a general description of the material to be used in gel filtration. The Office has alleged that the skilled person would have had a reasonable expectation of success that the materials described by Georgiou would have worked in Bondonc's gel filtration because Georgiou also uses the materials for gel filtration chromatography, as in Bondoc.

An analysis of obviousness of a claimed combination must include consideration of the results achieved by that combination. *The Gillette Co. v. S.C. Johnson & Son Inc.,* 16 USPQ2d 1923, 1928 (Fed. Cir. 1990). Critical to the analysis is an understanding of the particular results achieved by the new combination. *Id.* (citing *Interconnect Planning Corporation v. Feil,* 227 U.S.P.Q. 543, 551 (Fed. Cir 1985)).

Applicants submit that the alkyl dextran/methylene bisacrylamide matrix disclosed in Georgiou is taught for the purification by gel filtration chromatography of recombinant proteins produced in bacterial cells. The presently claimed method is directed to the production of infectious viral particles in eukaryotic cells, which is another technical field. There is no teaching in Bondoc or Georgiou, nor can any suggestion be inferred from the combination thereof, that dextran/methylene bisacrylamide matrix is suitable to purify adenovirus particles.

Indeed, as discussed in detail above, proteins and viral particles are quite distinct on numerous aspects including complexity, molecular weight, hydrophobicity and stability. All these parameters influence the adsorption properties of the molecule being purified to the chromatography material. It is clearly evident that the molecular weight of a protein is far distinct from that of a virus particle which exhibits at its surface hundreds of proteins. Moreover, the shape also plays a role in the retention of the molecule being purified to the gel filtration material. In this regard, the shape vary considerably, proteins are rather globular whereas adenovirus particles are icosahedric with rod-shaped fiber trimers protruding from the viral surface.

In view of the art-recognized differences between proteins and adenovirus particles, differences that influence the gel filtration separation, there is no reasonable expectation of success that the gel filtration material taught by Georgiou in connection with recombinant proteins would in fact work for purifying adenovirus particles. Moreover, the contaminants that one would seek to remove during purification are also different between bacterial and eukaryotic producer cells, and one may expect that these differences also contribute to the level of purity of the purified product.

As set forth above, there is no suggestion in this combination of references to use fluidized bed anion exchange and gel filtration chromatography using the specific chromatographic materials recited for purifying infectious adenoviral particles from a crude preparation. Shabram's mere inclusion of fluidized bed chromatography among other known techniques provides no suggestion to choose that method among all possible methods and has not been demonstrated even in rough experimentation, hence the mere inclusion in a listing not entitled to any presumption of operability. Further, in view of the art-recognized differences between macromolecules and adenovirus particles, one skilled in the art would not come to the conclusion that the fluidized bed chromatographic material disclosed by Berg and the gel filtration material disclosed by Georgiou would be chosen by a person of ordinary skill for purifying adenoviral particles, or even that the materials are applicable to the task. In short, there would have been no motivation to choose the presently claimed combination in the absence of impermissible hindsight. Furthermore, there is no evidence coming from the prior art suggesting a reasonable expectation of success in making the combination proposed by the Office, and substantial reason to doubt that the method of Berg could be successfully used in the presently claimed method.

For at least the foregoing reasons, the prior art fails to support a prima facie case of obviousness and accordingly, withdrawal of the rejection is proper.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned concerning such questions so that prosecution of this application may be expedited.

The Director is hereby authorized to charge any appropriate fees that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,

BUCHANAN INGERSOLL PC

Date: June 30, 2006

By: _____

Christopher L. North
Registration No. 50433

P.O. Box 1404
Alexandria, VA 22313-1404
703.836.6620